Home Office

NON-TECHNICAL SUMMARY

Nanotechnologies for cancer treatment, diagnosis and monitoring

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Nanotechnology, Nanomaterials, Therapy, Cancer, Glioblastoma

Animal types	Life stages
Mice	embryo, neonate, pregnant, adult, juvenile
Rats	adult, juvenile

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Nanomaterials are very small materials with at least one dimension (eg. width, length, height) that is typically less than 100 nanometres (one billionth of a metre) in size. The overall goal of this project is to design and test novel therapeutic, diagnostic and monitoring tools based on nanomaterials for applications in cancer.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Cancer remains an unmet clinical need that requires new approaches. The use of novel technologies based on nanomaterials (nanotechnologies) seeks to design smarter solutions with potential to overcome several key clinical problems. This includes delivery of therapeutic agents in more targeted and safer ways, use as medical imaging probes to aid clinical diagnosis and monitoring, and tools to identify biomarkers for early detection.

What outputs do you think you will see at the end of this project?

This project will generate new information on the application of nanotechnology in cancer. The new information gained from this project will be shared in the form of scientific publications, conference communications and through public engagement activities throughout this project.

While the timescale for these nanotechnologies to move from preclinical investigation to clinical products are more likely to be in the long term, we aim to build up rationale and justification within this project for clinical testing and validation of these technologies.

Who or what will benefit from these outputs, and how?

These communications will enable collaboration and stimulate further research into advanced biomedical nanotechnologies throughout the scientific community.

Eventually, patients will benefit from any effective new technologies for the diagnosis, monitoring or treatment of cancer that are developed based on the results obtained during this project.

How will you look to maximise the outputs of this work?

Publication and communication of our findings will always be the primary aim of this work. The goal of every experiment conducted under this licence will be to generate valid, high quality and therefore publishable results and we will endeavour to ensure that all findings meeting these criteria will be published (primarily in open access journals) to inform the wider scientific community, even if these do not support the therapeutic, diagnostic or monitoring potential of the particular nanomaterial, or nanomaterial device being investigated.

Species and numbers of animals expected to be used

- Mice: 5100
- Rats: 400

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are chosen for many of these studies for a variety of reasons, primarily as they are the least sentient mammalian species that will provide data applicable to humans. The tumour models using this species are already well characterised and validated with close similarities to the human tumours such that the findings and nanotechnologies developed have the best chance of clinical translation and population benefit. Rats are used as an alternative rodent model for some of this work where either the tumour models require this (eg. when using cancer cells of rat origin) or where the larger size of these rodents is beneficial for the nanotechnologies being developed (eg. as imaging agents).

The majority of research will use adult animals as the cancers being investigated are those which primarily affect adults.

Typically, what will be done to an animal used in your project?

For testing of nanomaterials in animals with cancer, tumours will be induced in these animals at an appropriate site for the relevant cancer (eg. under the skin for melanoma, in the brain for glioblastoma, in the lungs or other organs when modelling metastasis). For animals with brain tumours, an additional surgery may be performed to resect (surgically remove) the tumour, to mimic how these tumours are treated in patients.

To measure tumour growth in these animals either direct measurements with callipers (superficial tumours), or live imaging approaches (non-superficial tumours) may be used.

Either before, or during the course of tumour growth, animals will be administered with nanomaterials or a nanomaterial based therapy (either single dose or repeat administrations) or controls via appropriate route(s). The effects of the nanomaterials will be assessed by monitoring tumour growth using the above described methods in combination with other approaches such as imaging, taking

blood samples or collecting urine/faeces samples. We may also combine these nanomaterial therapies with existing therapies such as immunotherapy or tumour treating fields in order to design and develop strategies that could work synergistically with these approaches.

At the end of the experiment, usually before tumour growth is associated with any adverse clinical signs, animals will be humanely killed and tissues and tumours collected for further analysis.

What are the expected impacts and/or adverse effects for the animals during your project?

From our previous work, we don't expect the nanomaterials we use to have any particularly strong adverse effects. The majority of nanomaterials, or nanomaterial-based therapeutics will have been tested to confirm their biocompatibility in a companion project licence. The procedures used to administer the nanomaterials are usually the least invasive possible. Where surgical administration is needed, this will be done under anaesthesia and analgesia/additional support is provided to minimise the adverse effects associated with this.

Each of the tumour models used may have associated adverse effects. Superficial tumours are placed on sites (eg flank or back) that usually have minimal impact on the animals. For superficial tumours, skin ulceration may occur due to the rapid growth of the tumour or after nanomaterial treatment. These animals will be monitored closely and treated to avoid these ulcers progressing and causing suffering. If these ulcers start to worsen or bleed, animals will be humanely killed.

For metastatic tumours, the tumours may interfere with certain organ functions which is usually associated with weight loss which is used as a humane endpoint. Reparatory distress can occur, for example following the initial injection of cancer cells, but if this does not improve quickly (within minutes) animals would be humanely killed.

For tumours in the brain, animals usually behave normally but may lose weight or show subdued behaviour at the later stages of tumour growth. This is used as a humane endpoint to prevent any prolonged suffering.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mice: 100% moderate

Rats: 100% moderate

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Before materials or devices are tested in animals, they will first be tested in cell cultures, or in relevant in vitro biological systems to check the safety and to help determine the best doses that may be effective in vivo. Many responses to nanomaterials, including the pharmacology/biodistribution, are driven by complex interactions with multiple cell types as part of whole systems (eg. the immune system, the cardiovascular system etc). These cannot be effectively modelled and integrated in an in vitro setting and therefore require animal testing.

Due to the complex nature of the diseases we are investigating, it would not be possible to test the nanotechnologies we have developed without testing in animals. Cancer is a highly complex disease whereby uncontrolled growth of different populations of cancer cells interact closely with the immune system, generate new vasculature, remodel of the surrounding tissue/organ microenvironment, all of which need to be effectively modelled simultaneously to be representative of the patient populations. When considering the use of nanomaterials as therapeutics, targeting the tumour following administration in the blood stream can only be confirmed in animals with a complete circulatory system.

Which non-animal alternatives did you consider for use in this project?

In vitro cell cultures including 3D models and co-culture systems (up to and including organoids which are 3D in vitro models that contain multiple cell types and mimic organs more closely).

Why were they not suitable?

Non-animal alternatives such as those listed above can provide important information and are always used in the first instance for all new technologies and nanomaterials. This includes testing in organoid systems which is an ongoing effort by our group. However, none of these systems (including organoids) can effectively recapitulate the complex multi-system interactions of a whole organism, as is required for our objectives.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers of animals have been estimated in consultation with statisticians and based historical data from our own experiments using the same tumour models with similar nanomaterials or nanomaterial monitoring approaches.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We aim to use the minimum numbers of animals required to adequately and robustly address the research question. This has been determined with support from statisticians and use of rigorous experimental design considerations (as guided by the NC3Rs Experimental Design Assistant). Use of adequate numbers of animals will reduce variability, improve experimental consistency and confidence in outcomes. All assumptions on which sample size estimates are based will be re-evaluated once additional or new data is available from these studies and if necessary numbers of animals required will be revised for subsequent studies.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The main way we will reduce the number of animals we use will be to use longitudinal monitoring (eg. tumour growth measurements) and live imaging techniques. This will allow us to obtain data from the same animal over time instead of the more traditional method of killing a different animal at every timepoint. Where nanomaterials or nanomaterial therapies are being tested for the first time in animals, pilot studies will be run with smaller numbers to ensure safety and provide an initial assessment of efficacy or effect that will be used to statistically determine the correct number of animals to use for further investigations.

For breeding we will keep this to a minimum to maintain the transgenic line and provide only enough mice for our expected usage within a particular experiment or project. This will be regularly reviewed to prevent the unnecessary breeding and maintenance of genetically modified animals. Finally, at the end of each experiment we will collect as many tissues as possible in order to maximise the potential output from each experiment.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

A proportion of the experiments in this project will use superficial flank tumours. These are the least invasive and have minimal impact on the animal throughout the course of an experiment. The tumours

in these animals can also be visually monitored easily through physical measurement allowing any experiments to be terminated prior to the onset of any systemic clinical signs.

In some cases it will be necessary to use a systemic or metastatic model (eg. when developing a nanotechnology to detect or treat metastasis). For metastatic tumours, while these have more invasive properties they are not usually associated with significant clinical signs. This will be carefully monitored through the use of live imaging and continual (daily or more frequent) health monitoring.

For brain tumours, it is important that the tumour is growing in the brain to mimic what occurs in patients. The site of tumour induction is carefully planned such that tumours are induced in a site of the brain that is associated with minimal or no side effects associated with tumour growth. This also means that if the tumour is surgically removed, this is unlikely to be associated with any additional clinical signs.

In all models animals will be humanely killed before the onset of clinical signs that would exceed moderate suffering.

For administration of nanomaterials, the least invasive route that is relevant for the particular application will always be used. Where more invasive routes are necessary (eg. surgical administration) this will be scientifically justified and through proper aseptic technique, pain management and careful monitoring is not expected to cause any additional distress or prolonged suffering.

Why can’t you use animals that are less sentient?

The animals proposed are the least sentient mammalian species. The use of non-mammalian species (eg. Xenopus, Danio) would not be appropriate for the cancers or the clinical translation of the nanotechnologies under development. Mice and rats are the most appropriate for the work being carried out as they have circulatory, nervous and excretory systems very similar to humans, which allows us to model where the materials go, how the body breaks them down and how they are removed from the body in a system similar to humans. Importantly, the species used have well established cancer models that closely mimic human disease.

Adult animals are primarily used as they have the most complete development of the different systems that our nanotechnologies will interact with, including the brain, cardiovascular system, and immune system. Furthermore the cancers we are investigating are largely those that develop in adults such that it would not be clinically relevant to investigate these in more immature life stages.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Animals undergoing surgical procedures will receive appropriate analgesia to prevent any post-operative pain, will be carefully maintained at a suitable depth of anaesthesia and may also receive additional fluid support to prevent dehydration associated with longer procedures. These animals will be provided with additional husbandry such as mash/wet food, heated housing and careful monitoring in the immediate hours following surgery until normal activity is resumed.

Animals will be group housed and where animals have been individually housed for a particular purpose (post-surgical recovery) these will be grouped as soon as is appropriate.

Through our previous work we have refined animal monitoring approaches to minimise the harms to animals. Animals with more rapidly progressive disease (eg. later stages of cancer growth) will undergo frequent monitoring (up to twice daily) to identify and humanely kill any animals that may approach humane endpoints before the next monitoring timepoint.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

All experiments will be planned and executed with reference to the PREPARE and ARRIVE 2.0 guidelines to ensure effective experimental planning and proper reporting of experiments respectively. We will follow guidance from BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinements and LASA guiding principles for Administration of Substances and Aseptic surgery.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

The researchers working under this licence will be regularly encouraged to actively stay informed on advances in the 3Rs as is required by the conditions of their PIL. We will regularly check information on NC3Rs website and newsletters and we will attend institutional and regional 3Rs symposia. Any relevant advances, for example refinement of techniques or approaches, will be readily implemented into this project.